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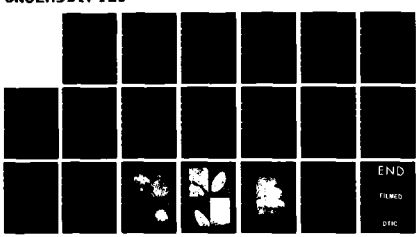

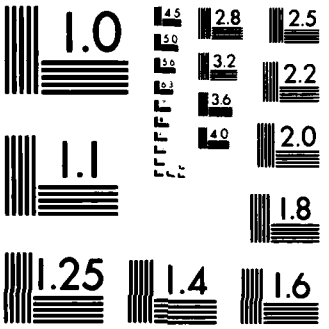

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Studies of the Biology of Phleboviruses in Sandflies

Annual Report

Robert B. Tesh, M.D.

February 1984

Supported by

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During the past year, laboratory colonies of three new sand fly species (Phlebotomus perniciosus, P. argentipes and P. Martini) were established. In addition, a continuous culture of Phlebotomus papatasi cells was also started. Attempts to clone the latter cell line have not been successful.

The growth of six representative phleboviruses (Toscana, Belterra, Naples, Arbia, Punta Toro and Pacui) was studied in sand flies following inoculation →

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and ingestion. The pattern after inoculation was similar; maximum virus titers were reached on the third or fourth day after infection and then seemed to stabilize at this level. In contrast, sand flies appeared to be fairly refractory to oral infection.

An attempt was made to develop a sand fly colony, chronically infected with a phlebovirus. Three virus-vector combinations were tried (Pacui virus and Lutzomyia longipalpis, Karimabad virus and P. papatasi, and Toscana virus with P. perniciosus). The Toscana - P. perniciosus combination gave the most interesting results. Transovarial infection rates in the F_1 and F_2 generations of these parenterally infected parent females was 30 and 41% respectively. Filial infection rates among F_2 generation adults from individual transovarially infected F_1 parents varied from 5 to 100%. Maintenance of Toscana virus in this chronically infected line appears to be by maternal transmission (cytoplasmic inheritance).

Ovaries of laboratory-reared P. papatasi, L. longipalpis and L. anthophora were examined to determine rates of autogenous and anautogenous oocyte development and to describe morphological changes in the ovarioles before and after ovulation. A high percentage of P. papatasi were autogenous; females of L. longipalpis and L. anthophora were not. Follicular relics were observed in all sand fly species studied and indicate that Polovodova's ovarian age-grading technique can be used for assessing gonotrophic age of sand flies.

Table of Contents

Subject	Page number
Summary	1
A. Brief History of Project	2-3
B. Sandfly Colonization	3
C. Experimental Infection of Sandflies	3-8
D. Studies of Follicular (Ovarian) Development in Sandflies	8-9
E. Sandfly Cell Cultures	9
F. Publications Resulting from or Supported by This Project	9-10
Table 1	11
Table 2	12
Table 3	13
Table 4	14
Table 5	15
Table 6	16
Table 7	17
Table 8	18
Table 9	19
Table 10	20
Table 11	21
Table 12	22
Table 13	23
Legends for Figures	24
Figure 1	25
Figure 2	26
Figure 3	27
Distribution List	28

Summary

During the past year, laboratory colonies of three new sand fly species (Phlebotomus perniciosus, P. argentipes and P. Martini) were established. In addition, a continuous culture of Phlebotomus papatasi cells was also started. Attempts to clone the latter cell line have not been successful.

The growth of six representative phleboviruses (Toscana, Belterra, Naples, Arbia, Punta Toro and Pacui) was studied in sand flies following inoculation and ingestion. The pattern after inoculation was similar; maximum virus titers were reached on the third or fourth day after infection and then seemed to stabilize at this level. In contrast, sand flies appeared to be fairly refractory to oral infection.

An attempt was made to develop a sand fly colony, chronically infected with a phlebovirus. Three virus-vector combination were tried (Pacui virus and Lutzomyia longipalpis, Karimabad virus and P. papatasi, and Toscana virus with P. perniciosus. The Toscana - P. perniciosus combination gave the most interesting results. Transovarial infection rates in the F_1 and F_2 generations of these parenterally infected parent females was 30 and 41% respectively. Filial infection rates among F_2 generation adults from individual transovarially infected F_1 parents varied from 5 to 100%. Maintenance of Toscana virus in this chronically infected line appears to be by maternal transmission (cytoplasmic inheritance).

Ovaries of laboratory-reared P. papatasi, L. longipalpis and L. anthophora were examined to determine rates of autogenous and anautogenous oocyte development and to describe morphological changes in the ovarioles before and after ovulation. A high percentage of P. papatasi were autogenous; females of L. longipalplis and L. anthophora were not. Follicular relics were observed in all sand fly species studied and indicate that Polovodova's ovarian age-grading technique can be used for assessing gonotrophic age of sand flies.

A. Brief History of the Project

This research project began on 1 September 1980. For the first two years, it was funded as contract DAMD17-80-C-0178, entitled "Studies on the Transovarial Transmission of Phlebotomus Fever Viruses in Sandflies." The original contract terminated on 30 September 1982 and a second contract (DAMD 17-83-C-3002), entitled "Studies on the Biology of Phleboviruses in Sandflies", began on 1 October 1982. The second contract terminated on 30 September 1983. On 1 October 1983, the project was refunded under a grant (DAMD17-83-G-9561) with the same title, "Studies on the Biology of Phleboviruses in Sandflies." This annual report covers work done in 1983-84 under the grant (DAMD17-83-G-9561); however, it should be noted that this is actually the fourth year of work on the project, since the overall objectives and personnel have changed little during this four year period.

During the first 16 months of the project (Sept.1980 - Dec.1981), our efforts were focused primarily on establishing laboratory colonies of phlebotomine sandflies. This work took much longer than we had anticipated; and, in retrospect, our original timetable was unrealistic. We found, as many other investigators before us have found, that sandflies are difficult to rear in captivity. These insects are much less prolific than mosquitoes and each generation takes approximately 6 to 8 weeks to develop. Furthermore, the daily care of sandflies is extremely labor intensive. Therefore, new techniques for rearing the insects had to be developed. For these reasons, it was not until December of 1981 that we finally had sandfly colonies of sufficient size and productivity to actually begin experimental studies. We are now able to mass rear sandflies and produce several thousand insects of each species per generation. In fact, our production now exceeds our experimental needs.

Since January 1982, most of our work has been experimental studies of the

behavior of various phleboviruses in laboratory-reared sandflies. In addition, two new continuous sandfly cell lines were established. Some of this work was reported in the previous annual report dated 1 February 1983. The present report describes studies done during the past year and attempts to correlate them with our earlier studies and proposed future work.

B. Sandfly Colonization

During the past year, colonies of four new sandfly species were established in our laboratory. These were Phlebotomus perniciosus obtained from Dr. M. Maroli, Istituto Superiore di Sanita, Rome, Italy; Phlebotomus longipes, collected near Addis Ababa, Ethiopia and brought to us by Dr. F. A. Neva, National Institutes of Health; Phlebotomus argentipes, collected near Sagar, Karnataka State, India, by G. B. Modi, Yale University; and Phlebotomus martini, obtained from Dr. Ray Beach, U.S. Army Medical Research Unit, Kenya. With the exception of P. martini, these colonies are well established and prolific.

In addition to the aforementioned species, we also have well established colonies of the following sandflies: Phlebotomus papatasi (geographic strains from India, Israel and Egypt), Lutzomyia longipalpis and Lutzomyia anthophora. During the coming year, we hope to start colonies of several other Neotropical species (Lutzomyia spp.) through contacts in Panama, Colombia and Brazil.

During the past year, various numbers of sandflies (eggs, larvae or adults) from our colonies were supplied to scientists at 8 institutions in the United States and England. These are identified in Table 1.

C. Experimental Infection of Sandflies

To date, we have studied the behavior of 14 phleboviruses (Rio Grande, Pacui, Naples, Punta Toro, Karimabad, Sicilian, Saint Floris, Rift Valley fever, Gabek Forest, Itaituba, Salehabad, Arbia, Toscana and Belterra) in one or more

sandfly species following oral and/or parenteral infection. Our results are summarized below:

1. Most of these phleboviruses replicate in sandflies after intrathoracic inoculation, although the level of replication of a given virus varies in different sandfly species. For example, Table 2 shows the growth of Toscana virus in P. perniciosus, P. papatasi and L. longipalpis following inoculation. The virus replicated in all three species, but highest titers were obtained in P. perniciosus, the presumed natural vector.

Table 3 shows the growth of Belterra virus in L. longipalpis after inoculation. In this experiment, the titer of the virus inoculum was quite high ($\pm$ $10^{3.3}$); since the virus persisted at this level for 7 days, it is uncertain whether multiplication actually occurred. Belterra virus is of special interest because of its close antigenic relationship with Rift Valley fever virus. Belterra virus was not transovarially transmitted by infected L. longipalpis.

Tables 4 - 10 show the growth of six representative phleboviruses and one vesiculovirus in sandflies after inoculation. The pattern with each of the phleboviruses was similar; maximum virus titers were reached on the third or fourth day after inoculation and then the viruses seemed to stabilize in the insects. This has been the pattern observed in almost all of the viruses tested.

2. In contrast to the results obtained by parenteral infection, sandflies appear to be fairly refractory to oral infection. Tables 4-10 show the growth of six representative phleboviruses and one vesiculovirus (Carajas) in sandflies after ingestion. With 4 of these virus (Naples, Arbia, Punta Toro and Carajas), no replication occurred after ingestion. Despite relatively high virus titers in the insects immediately post-feeding, by the third or fourth day, no virus

could be detected. The results obtained with Pacui, Gabek Forest and Toscana viruses (Tables 7-9) are more difficult to interpret. High titers of virus were detected in all of the sandflies tested immediately after feeding. Then during the third, fourth and fifth days post-feeding, virus titers appeared to drop and in some cases were undetectable. However, on the six and seventh days, some of the insects sampled again contained fairly high amounts of virus. Since the insects were not sampled beyond the seventh post-feeding day, it is uncertain whether these results indicate an extremely long eclipse phase in the sandflies or whether it means that only some of the insects are susceptible to oral infection. Because of the results obtained with Naples, Arbia and Punta Toro viruses (Tables 4-6), as well as the relatively high titers of virus ($10^{3.0}$-$10^{4.2}$) observed in orally infected flies on the sixth and seventh days after feeding (Tables 7-9) we suspect the latter.

A second bit of evidence also suggests that ingestion of viruses is not an efficient method for infecting sandflies. Following inoculation with Toscana virus (Table 9), Phlebotomus perniciosus females transmitted the agent transovarially to 30% of their F_1 progeny in the first gonotrophic cycle. In contrast, no virus was recovered from 139 F_1 offspring from the first ovarian cycle or from 21 offspring from the second ovarian cycle of female P. perniciosus ingesting Toscana virus (Table 9). We have not yet tested whether orally infected flies can transmit virus by bite to susceptible animals.

3. Experimental transovarial transmission has been demonstrated with 7 of 13 phleboviruses tested (Table 11). Infection rates in the F_1 progeny from experimentally infected female parents varied from 1.5 to 60.0%. All cases of transovarial virus transmission occurred in parenterally infected females. However, it should be noted that transovarial transmission did not occur with

all phleboviruses or with all sandfly species tested. As shown in Table 11, we were unable to demonstrate transovarial transmission of Gabek Forest, Punta Toro, Rift Valley fever, Naples, Salehabad or Belterra viruses in sandflies. Furthermore, while Toscana virus was transovarially transmitted to 30% of the F_1 progeny from experimentally infected P. perniciosus, this agent was not transmitted to any of the progeny of parenterally infected P. papatasi or L. longipalpis. Toscana virus has been recovered repeatedly from both male and female P. perniciosus in nature, whereas it has never been associated with P. papatasi or L. longipalpis. Likewise, Pacui virus was transmitted to 33% of the F_1 progeny of experimentally infected L. longipalpis but to only 2% of the offspring of infected P. papatasi.

The above results plus the repeated recoveries of phleboviruses from field-collected male sandflies suggest that some of these agents are maintained in nature in part, or perhaps entirely, by transovarial transmission. In an attempt to test this hypothesis, we began to study the mechanism of phlebovirus transovarial transmission and to determine if some of these viruses could be maintained from generation to generation in a sandfly population by transovarial transmission alone.

Three different phleboviruses and sandfly species were used in these studies: Pacui virus in L. longipalpis, Karimabad in P. papatasi, and Toscana in P. perniciosus. Initially, infection rates were determined in the F_1 offspring of females experimentally infected with each of these viruses. The results are shown in Table 12. The F_1 females were fed on clean hamsters to induce egg formation and then their progeny (the F_2 generation) were also tested. Only the F_2 offspring of transovarially infected F_1 females were examined. With Pacui virus, 32% of the F_1 progeny were infected but only 6.8%

of the F_2 offspring from F_1 positive females contained virus. Thus the percentage of infected offspring dropped in the F_2 generation. Similar results were obtained with Karimabad virus. Sixty percent of the F_1 offspring were infected, but only 8.8% of their progeny (F_2 generation) contained virus.

Toscana virus, however, gave somewhat different results (Table 12). Thirty percent of the F_1 progeny from experimentally infected P. perniciosus female parents contained virus. Of 248 offspring (F_2) produced by 14 of the transovarially infected F_1 females, 41% contained virus. Thus the percentage of Toscana infected offspring actually increased in the F_2 generation. Table 13 shows the filial infection rates in the F_2 progeny from the 14 transovarially infected F_1 females. Filial infection rates in these females varied from 4.9 to 100%. The significant finding here is that some of the infected F_1 females transmitted virus to almost all of their progeny! This experiment was not carried beyond the F_2 generation, but we intend to repeat it and to follow infection rates in subsequent generations.

The results shown in Table 13 are very similar to those described earlier with sigma, Matsu, San Angelo and California encephalitis viruses in their respective insect hosts. Each of the latter four viruses is transovarially transmitted in its arthropod vector by a mechanism known as "cytoplasmic inheritance" or the "stabilized condition". If cytoplasmic inheritance also occurs with some of the phleboviruses, it provides a mechanism for these agents to be maintained in the vector population during adverse climatic conditions and in the absence of susceptible vertebrate hosts. This also fits with previous findings that many of the phleboviruses produce a very low level and transient viremia in mammals and that sandflies are relatively refractory to oral infection with these viruses. Additional studies are now in progress to

determine if Toscana virus and other phleboviruses are transmitted by cytoplasmic inheritance in sandflies.

D. Studies of Follicular (Ovarian) Development in Sandflies

This work was done in collaboration with Dr. L. A. Magnarelli, Department of Entomology, The Connecticut Agricultural Experiment Station. Ovaries of laboratory-reared P. papatasi, L. longipalpis and L. anthophora were examined to determine rates of autogenous and anautogenous oocyte development and to describe morphological changes in the ovarioles before and after ovulation. Oneof the objectives of this work was to learn more about follicular development in sandflies, so that we can examine ovaries of infected females by immunofluorescence and/or electron microscopy and better understand the mechanism of transovarial virus transmission. Work to date has only been done with normal (uninfected) females from our laboratory colonies.

Female P. papatasi from two distinct geographic regions (India and Israel) produced eggs autogenously within 6 days after eclosion. If suitable vertebrate hosts were available, these insects would also ingest blood during the first gonotrophic cycle. This observation supports previous reports that sandflies will take blood repeatedly during a single gonotrophic cycle. This characteristic (multiple feedings from different vertebrate hosts) increases their vector potential. Likewise, the ability to produce eggs autogenously would insure the survival of a transovarially transmitted pathogen in the absence of susceptible vertebrate hosts for the insect to feed upon.

In contrast to P. papatasi, females of L. longipalpis and L. anthophora were not autogenous. These species only developed eggs 3-6 days after a blood meal. Follicular relics were observed in all sandfly species studied and indicate that Polovodova's ovarian age-grading technique can be used for

assessing gonotrophic age of sandflies. The various stages of follicular development were observed and photographed and a manuscript was prepared (now in press, J. Med. Entomology) describing our findings. Figures 1-3 show representative photographs of various stages of follicular development in sandflies.

E. Sandfly Cell Cultures

In previous reports, we described two continuous cultures of sandfly cells which were started from eggs of Lutzomyia longipalpis and Phlebotomus papatasi. These are the first sandfly cell lines. Both were tested for their susceptibility to infection with a variety of phleboviruses. To our disappointment, neither of these cell lines was very susceptible to infection with phleboviruses. A brief attempt was made to clone the Phlebotomus papatasi cell line in hopes that a susceptible cell population could be identified and cultured. However, to date we have been unable to clone the cells and this project has been largely abandoned.

F. Publications resulting from or supported by this project

Tesh, R.B. and Modi, G.B. Growth and transovarial transmission of Chandipura virus (Rhabdoviridae: Vesiculovirus) in Phlebotomus papatasi. **Am. J. Trop. Med. Hyg.** 32:621-623, 1983.

Tesh, R.B. and Modi, G.B. Development of a continuous cell line from the sand fly, Lutzomyia longipalpis (Diptera: Psychodidae), and its susceptibility to infection with arboviruses. **J. Med. Ent.** 20:199-202, 1983.

Modi, G.B. and Tesh, R.B. A simple technique for mass-rearing Lutzomyia longipalpis and Phlebotomus papatasi (Diptera: Psychodidae) in the laboratory. **J. Med. Ent.** 20:568-569, 1983.

Endris, R.G., Tesh, R.B. and Young, D.G. Transovarial transmission of Rio Grande virus (Bunyaviridae: Phlebovirus) by the sand fly, Lutzomyia anthophora. **Am. J. Trop. Med. Hyg.** 32:862-864, 1983.

Travassos, A.P.A., Tesh, R.B., Pinheiro, F.P., Travassos da Rosa, J.F.S. and Peterson, N.E. Characterization of eight new phlebotomus fever serogroup arboviruses (Bunyaviridae: Phelebovirus) from the Amazon Region of Brazil. **Am. J. Trop. Med. Hyg.** 32:1164-1171, 1983.

McMahon Pratt, D., Modi, G. and Tesh, R.B. Leishmania mexicana stage specific antigens found on promastigotes of infected Lutzomyia longipalpis. **Am. J. Trop. Med. Hyg.** 32:1268-1271, 1983.

Tesh, R.B. Undifferentiated arboviral fevers. In **Tropical and Geographical Medicine.** K.S. Warren and A.A.F. Mahmond (editors). McGraw-Hill Book Co., New York, 1984, p.660-666.

Tesh, R.B. Transovarial transmission of arboviruses in their arthropod vectors. In **Current Topics in Pathogen-Vector-Host Research.** K.G. Harris (editor). Praeger Scientific, New York 2: 57-76, 1984.

Magnarelli, L.A., Modi, G.B. and Tesh, R.B. Follicular development and parity in phlebotomine sand flies (Diptera: Psychodidae). **J. Med. Ent.** 21: 681-689, 1984.

Table 1

Recipients of Sandflies Supplied Under Grant DAMD-17-83-G-9561

Recipient	Species supplied
Louis C. Rutledge Division of Cutaneous Hazards Letterman Army Institute of Research San Francisco, California 94129	*Phlebotomus papatasi*
Major Peter V. Perkins, Ph.D. Department of Entomology Walter Reed Army Institute of Research Washington, D. C. 20307	*Phlebotomus papatasi* *Phlebotomus perniciosus* *Phlebotomus argentipes*
Capt. Alfred L. Hoch, Ph.D. Department of Arboviral Entomology U.S.Army Research Institute of Infectious Diseases Fort Detrick, Frederick, Maryland 21701	*Phlebotomus papatasi* *Lutzomyia anthophora*
Dr. Richard G. Endris Plum Island Animal Disease Center U. S. Department of Agriculture Greenport, New York 11944	*Lutzomyia anthophora*
Mr. Moises Montoya Division of Vector-Borne Viral Diseases Center for Disease Control Fort Collins, Colorado 80522	*Lutzomyia anthophora*
Dr. Robert Killick-Kendrick Department of Entomology Imperial College London, England	*Phlebotomus papatasi*
Dr. Richard D. Kreutzer Department of Biology Youngstown State Universtiy Youngstown, Ohio 44555	*Phlebotomus papatasi* *Phlebotomus perniciosus* *Lutzomyia longipalpis* (frozen specimens for isoenzyme studies)
Dr. Diane McMahon Pratt Division of Tropical Medicine Harvard Medical School Boston, Massachusetts 02115	*Phlebotomus papatasi* *Lutzomyia longipalpis* (frozen specimens infected with *Leishmania*)
Dr. John Edman Department of Entomology University of Massachusetts Amherst, Massachusetts 01003	*Phlebotomus papatasi* *Lutzomyia longipalpis* (Preserved specimens in 70% alcohol)

Table 2

Growth of Toscana virus in P. perniciosus, P. papatasi, and L. longipalpis after intrathoracic inoculation

Day post-inoculation	P. perniciosus		P. papatasi		L. longipalpis	
	Range of virus titers in pos. flies*	Mean virus titer	Range of virus titers in pos. flies*	Mean virus titer	Range of virus titers in pos. flies*	Mean virus titer
0	2.0 - 2.7	2.2	1.4 - 2.4	1.9	1.6 - 2.7	2.2
1	2.0 - 3.0	2.6	2.0 - 2.8	2.5	1.8 - 2.6	2.1
2	2.5 - 3.7	3.0	1.7 - 2.9	2.1	2.0 - 2.7	2.3
3	3.2 - 4.2	3.9	2.3 - 3.2	2.8	1.7 - 2.2	2.0
4	3.2 - 4.7	3.7	2.5 - 3.3	2.9	2.2 - 2.6	2.4
5	3.0 - 4.2	3.6	2.4 - 3.0	2.8	1.7 - 2.6	2.4
6	3.8 - 4.7	4.2	2.0 - 3.0	2.6	2.5 - 3.5	2.9
7	3.0 - 4.0	3.7	-	-	2.4 - 3.2	2.6

* Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 3

Growth of Belterra virus in Lutzomyia longipalpis after intrathoracic inoculation

Day post-inoculation	Range of virus titers in in infected flies*	Mean virus titer*
0	3.0 - 3.7	3.3
1	2.5 - 2.7	2.6
2	2.8 - 3.2	3.1
3	2.8 - 3.6	3.4
4	2.0 - 3.0	2.6
5	2.6 - 3.4	2.8
6	3.2 - 3.4	3.3
7	2.6 - 3.7	3.3

* Virus titers expressed as $\log_{10}$ of plaque units per insect. Five sand flies were sampled each day.

Table 4

Comparative growth of Naples virus in Phlebotomus papatasi following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.0,2.0,2.5,2.7**	2.0, 2.6, 2.6, 2.9, 3.2
1	2.0,2.2,2.2,2.4,2.6	1.7, 2.0, 2.6, 2.6, 2.8
2	2.8,2.9,3.0,3.3,3.4	<0.7, 1.0, 2.2, 2.2, 2.3
3	3.0,3.5,3.6,4.2,4.2	<0.7,<0.7, 0.7, 0.7, 1.2
4	3.0,3.0,3.3,3.7,4.3	<0.7,<0.7,<0.7,<0.7,<0.7
5	3.0,3.4,3.6,4.0,4.0	<0.7,<0.7,<0.7,<0.7,<0.7
6	4.0,4.0,4.0,4.2,4.2	<0.7,<0.7,<0.7,<0.7,<0.7
7	3.8,4.0,4.2,4.4,4.5	<0.7,<0.7,<0.7,<0.7,<0.7

* Insects were fed on a mixture of infected newborn mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 5

Comparative growth of Arbia virus in Phlebotomus perniciosus following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	1.2,1.2,1.4,1.6,1.6**	2.7,2.8,2.8,2.8,3.2
1	<1.7,<1.7,<1.7,<1.7,<1.7	2.0,2.0,2.4,2.8,
2	2.2,2.5,3.0,3.0,3.4	(Not Tested)
3	3.0,3.0,3.0,3.3,3.8	<0.7,<0.7,<0.7,<0.7,<0.7
4	2.0,3.0,3.4,3.5,3.7	<0.7,<0.7,<0.7,<0.7,<0.7
5	3.7,3.7,4.0,4.2,4.4	<0.7,<0.7,<0.7,<0.7,<0.7
6	2.5,3.2,3.6,3.7,	<0.7,<0.7,<0.7,<0.7,<0.7

* Insects were fed on a mixture of infected mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 6

Comparative growth of Punta Toro virus in Lutzomyia longipalpis following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.0,2.2,2.3,2.3**	2.4, 2.8, 2.8, 3.9, 4.2
1	1.7,1.7,2.0,2.9,3.2	2.0, 2.8, 3.0, 3.0, 3.3
2	3.0,3.0,3.4,3.6,3.8	<0.7,<0.7,<0.7, 0.7, 0.7
3	3.9,4.0,4.0,4.2,4.2	<0.7,<0.7, 0.7, 1.3, 1.7
4	3.7,3.7,4.0,4.4,4.4	<0.7,<0.7,<0.7,<0.7, 0.7
5	3.5,3.9,4.0,4.3,4.6	<0.7, 0.7, 1.0, 1.0, 1.4
6	4.0,4.2,4.2,4.3,4.7	<0.7,<0.7,<0.7,<0.7, 0.7
7	4.0,4.3,4.6,4.6,4.6	(Not tested)

* Sandflies were fed on a hamster infected with Punta Toro virus. Titer of hamster's viremia = $10^{7.8}$ PFU/ml.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 7

Comparative growth of Pacui virus in Lutzomyia longipalpis following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.2,2.4,2.5,2.8**	3.2,3.4,3.5,3.6,3.9
1	4.0,4.0,4.0,4.0,4.8	<0.7,<0.7,<0.7,<0.7,<0.7
2	4.6,4.7,5.0,5.2,5.4	<0.7,<0.7,<0.7,<0.7, 0.7
3	5.2,5.2,5.4,5.5,5.6	<0.7,<0.7, 0.7, 1.0, 1.7
4	4.8,5.0,5.4,5.5,5.7	(Not tested)
5	5.0,5.2,5.2,5.3,5.4	<0.7,<0.7,<0.7,<0.7,<0.7
6	5.0,5.2,5.2,5.3,5.5	<0.7,<0.7,<0.7, 3.0, 3.4
7	4.0,4.8,5.0,5.0,5.6	<0.7,1.6,3.4,3.8
8	4.9,5.0,5.0,5.4,5.4	(Not tested)

* Insects were fed on a mixture of infected newborn mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 8

Comparative growth of Gabek Forest virus in Phlebotomus papatasi following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	1.0,1.7,2.2,2.2,2.5**	4.3,4.5,4.5,4.8,4.8
1	2.3,2.6 (only 2 tested)	4.2,4.5,4.9,5.0,5.0
2	2.7,3.0,3.2,3,2	<0.7,<0.7, 1.0,3.0
3	4.0,4.0,4.0,4.2,4.5	<0.7,<0.7,<0.7,2.4,3.7
4	4.0,4.8,5.0,5.0,5.5	<0.7,<0.7, 2.9,3.7,4.9
5	4.6,4.8,4.9,5.0,5.2	<1.7,2.0,2.0,3.2,3.7
6	4.6,4.9,5.2,5.3,5.3	<1.7,<1.7,<1.7,<1.7,<1.7
7	4.8,5.0,5.0,5.2,5.3	<1.7,<1.7,2.5,4.0,4.2

* Insects were fed on a hamster infected with Gabek Forest virus. Titer of hamster's blood = $10^{9.0}$ PFU/ml.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 9

Comparative growth of Toscana virus in Phlebotomus perniciosus following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.0,2.2,2.2,2.7**	3.2,3.4,3.5,3.6,3.8
1	2.0,2.3,2.5,3.0,3.0	2.9,3.4,3.7,3.7,3.9
2	2.5,2.9,3.0,3.2,3.7	1.7,1.8,1.9,2.3,3.0
3	3.2,4.0,4.0,4.2,4.2	1.3,1.5,2.0,2.0,2.8
4	<1.7,2.8,3.2,4.2,4.7	<0.7,<0.7,<0.7, 1.0,3.0
5	3.0,3.3,3.7,4.0,4.2	<0.7,<0.7,<0.7, 2.7,2.8
6	3.8,4.0,4.0,4.3,4.7	2.8,2.9,3.0,3.2,3.7
7	3.0,3.8,3.8,3.9,4.0	<0.7,3.5,3.6,4.0

* Insects were fed on a mixture of infected mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 10

Comparative growth of Carajas virus (Rhabdoviridae: Vesiculovirus) in Lutzomyia longipalpis following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	1.4,1.7,1.8,1.8,1.9**	3.0,3.0,3.2,3.3,3.4
1	3.0,3.3,3.4,3.5,3.6	2.3,2.6,2.6,2.7,2.7
2	2.8,3.8,3.8,3.9,4.0	<0.7, 1.2,1.2,1.2,1.4
3	3.3,3.8,4.0,4.0,4.4	<0.7,<0.7,<0.7,<0.7,3.0
4	4.3,4.8,5.0,5.0,5.3	<0.7,<0.7,<0.7,<0.7,<0.7
5	4.6,5.0,5.0,5.0,6.0	<0.7,<0.7,<0.7,<0.7,<0.7
6	4.4,4.9,5.2,6.0,6.2	<0.7,<0.7,<0.7,<0.7,<0.7
7	4.0,4.5,4.5,4.9,5.2	<0.7,<0.7,<0.7,<0.7,<0.7

* Insects were fed on a mixture of infected mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 11

Transovarial transmission rates of selected phleboviruses in sandflies

Virus used to infect parents*	Sandfly species	Number of F_1 progeny tested	Percentage of F_1 progeny infected
Rio Grande	Lutzomyia anthophora	62	54.8
Pacui	Lutzomyia longipalpis	122	32.0
Pacui	Phlebotomus papatasi	51	2.0
Sicilian	Phlebotomus papatasi	135	1.5
Gabek Forest	Phlebotomus papatasi	50	0
Punta Toro	Lutzomyia longipalpis	100	0
Karimabad	Phlebotomus papatasi	220	60.0
Rift Valley fever	Phlebotomus papatasi	235	0
Saint Floris	Phlebotomus papatasi	112	6.3
Salehabad	Phlebotomus papatasi	94	0
Toscana	Phlebotomus perniciosus	146	30.1
Toscana	Phlebotomus papatasi	117	0
Toscana	Lutzomyia longipalpis	240	0
Arbia	Phlebotomus perniciosus	87	20.7
Belterra	Lutzomyia longipalpis	60	0
Naples	Phlebotomus papatasi	179	0

*Female parents in these experiments were infected by inoculation.

Table 12

Transovarial transmission rates in offspring of female sandflies inoculated with 3 different phleboviruses

	Sandfly species - Virus type		
	L. longipalpis - Pacui	*P. papatasi* - Karimabad	*P. perniciosus* - Toscana
F_1 generation	39/122 (32.0%)	132/220 (60.0%)	44/146 (30.1%)
F_2 generation	7/103 (6.8%)	13/147 (8.8%)	102/248 (41.1%)

Table 13

Toscana virus filial infection rates among progeny (F_2 generation) from transovarially infected F_1 female *P. perniciosus*

Female No.	No. infected/Total offspring /tested	Filial infection rate (%)
169861	2/41	4.9
169870	2/34	5.9
169825	5/31	16.1
169889	1/5	20.0
169849	7/28	25.0
169885	2/3	66.7
169892	13/19	68.4
169830	8/11	72.7
169853	18/24	75.0
169827	13/17	76.5
169876	10/12	83.3
169879	16/18	88.9
169828	2/2	100
169820	3/3	100
TOTAL	102/248	41.1

Legends for Attached Figures

Fig. 1. Primary follicles in L. longipalpis. (a) Stage I with nurse cells (arrow), 1500 X. (b) Stage II, 1500 X. (c) Oocytes with different amounts of yolk, 750 X. (d) State III, 750 X.

Fig. 2. Oocytes in L. longipalpis. (a) Electron micrograph of 2 adjacent ovarioles showing follicular epithelial cells (arrows), 4100 X. (b) Early state IV isolated from ovarioles, 375 X. (c) Stage V primary follicle and stage I secondary follicle (arrow), 750 X. (d) Sac-stage follicular tube with concentration of cellular debris (arrow), 750 X.

Fig. 3. Ovariole of parous L. longipalpis with coalesced follicular relic (arrow) 900X.

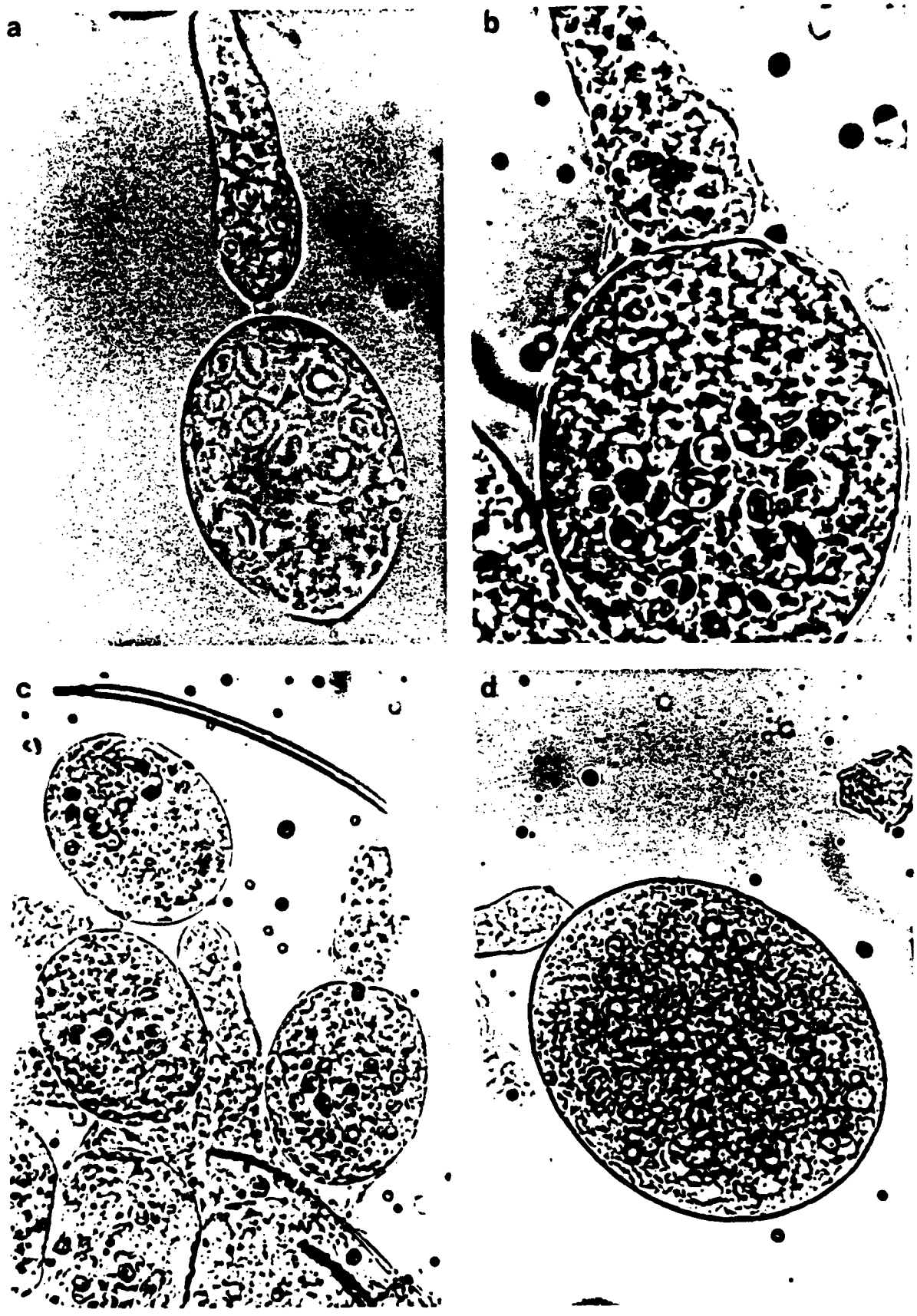

FIGURE 1

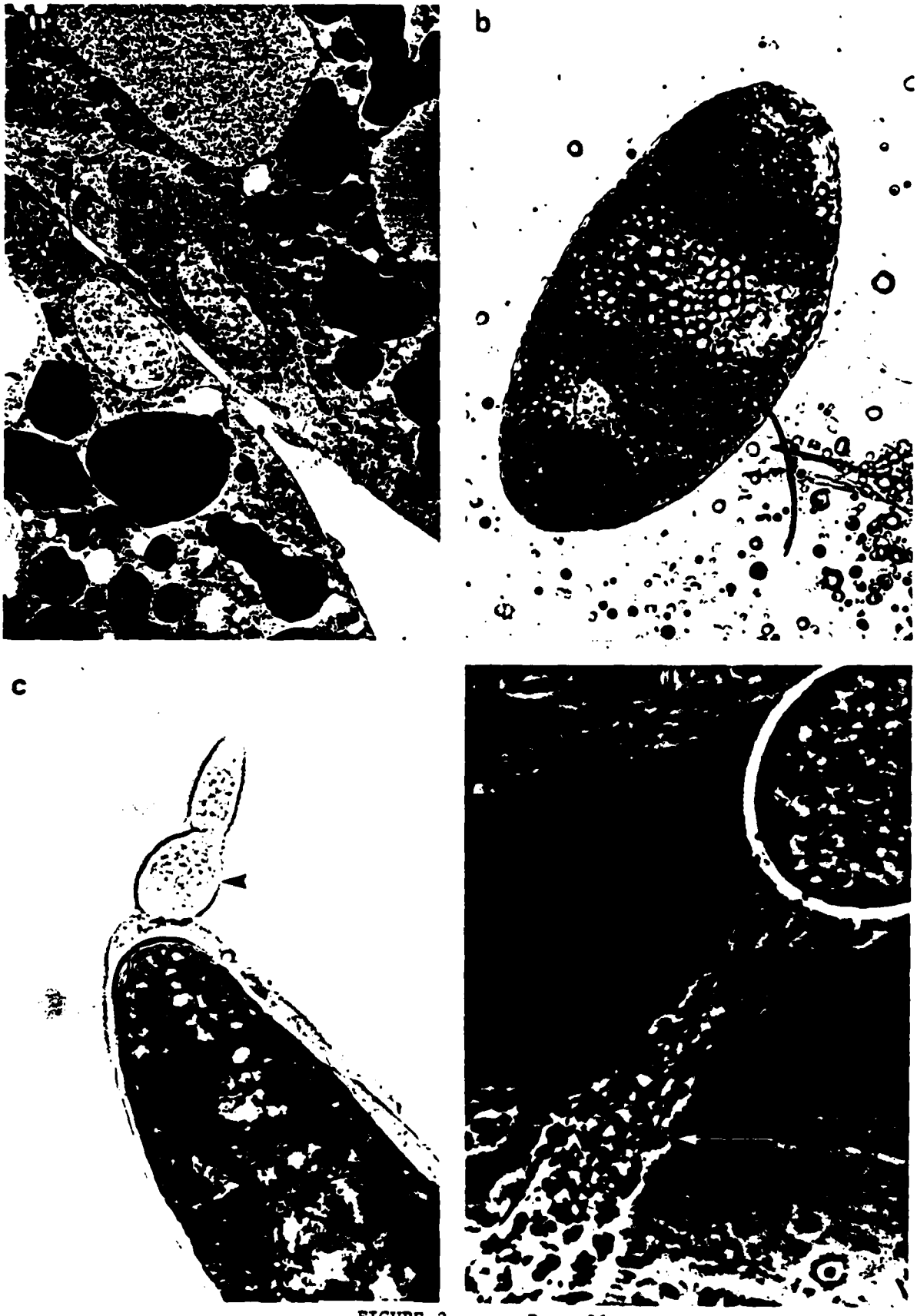

FIGURE 2

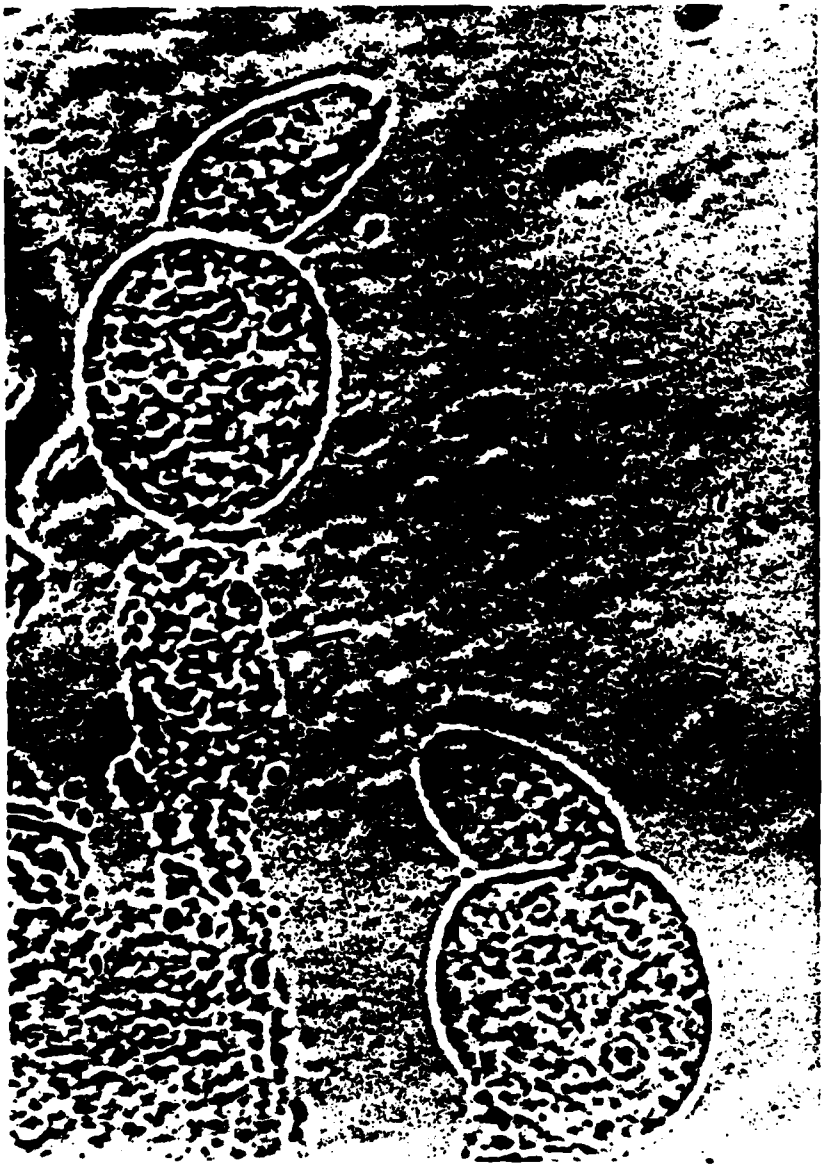

FIGURE 3 Page 27

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